Cell, Vol. 89, 159–161, April 18, 1997, Copyright ©1997 by Cell Press

Bone Morphogenesis and Modeling: Soluble Signals Sculpt Osteosomes in the Solid State

Minireview

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It is common knowledge that bone, like all matter, is in both a soluble and a solid state. There is a continuum between the soluble and solid states that is regulated by signals in solution interacting with insoluble extracellular matrix. A beautiful example of the interface of signals and extracellular matrix is the human skeleton. The skeleton evolved for several purposes: for locomotion to forage for food in the wild; for copulation to procreate the species and maintain the gene pool; for protection of vital internal organs including hematopoietic marrow; and as a reservoir of vital ions such as calcium and phosphate and of trace elements such as magnesium and zinc with critical roles in metabolism including DNA synthesis, replication, repair, transcriptional and translational regulation, and finally (the inevitable) cell death and apoptosis.

Bone can develop by one of two routes. Mesenchymal cells can differentiate directly to bone, as occurs in the flat bones of the craniofacial skeleton; this process is termed intramembranous ossification. Alternatively, cartilage can provide a blueprint or template for bone morphogenesis, as occurs in the majority of the over 200 bones in the human skeleton. The cartilage blueprint is ephemeral and is replaced by bone in a process termed endochondral ossification (Reddi, 1981). Bone is also continuously modeled during growth and development and remodeled throughout the life of the organism in response to physical and chemical signals. The physical signals include, but are not limited to, gravity, electricity, magnetism, and ultrasound.

Bones are a superb example of design architecture and engineering. They consist of cortical or compact bone and cancellous or trabecular bone. The outer cortical and inner trabecular bone network permits mechanical function and adaptation to changing mechanical environment and signals. Osteoporosis is a systemic skeletal disease of low bone mass, loss of the network of trabeculae, and deterioration of microarchitecture in bone trabeculae especially in postmenopausal women (Riggs and Melton, 1992). In developing bone, osteoblasts of mesenchymal lineage initially form woven bone that is subsequently modeled by osteoclasts of the hematopoietic monocyte-macrophage lineage (Marks, 1989), leading to the formation of lamellar bone with its characteristic concentric rings with a central blood vessel. The osteocytes and osteoblasts in the bone form a syncytium by gap junctions and connecting canalicular cell processes (Vukicevic et al., 1990). The primordial signals for the lineage of bone-forming osteoblasts are the family of bone morphogenetic proteins (Reddi, 1997).

The newly formed bone, both cortical and cancellous, consists of extracellular matrix (Piez and Reddi, 1984) with a constellation of constituents (Table 1) that is mineralized. Bone is akin to reinforced concrete. The fibrillar collagens with associated supramolecular assembly of a cornucopia of noncollagenous proteins (Table 1) are encrusted with the common geomineral hydroxyapatite with the generic structure of 3 $Ca_3(PO_4)_2 \cdot Ca(OH)_2$. The hydroxyapatite in vivo is associated with carbonate, fluoride, magnesium, and citrate. The bone mineral has profound avidity for ''bone-seeking'' substances such as tetracyclines and bisphosphonates (during peace time, thanks to physicians) and uranium and plutonium (during war, thanks to generals!). This property of mineral-associated tetracyclines allows one to mark and determine bone apposition rates using various fluorochromes. The bone mineral is opaque to X-rays, permitting orthopedic surgeons and radiologists to image the skeleton. How is the bone modeled in the embryo and remodeled in the adult? This question is addressed by an exciting report in this issue of *Cell* by Simonet et al. describing a novel secreted member of the TNF receptor superfamily increasing bone density and is the focus of this minireview.

The formation of bone by osteoblasts, and its modeling in a growing child and remodeling in adults by osteoclasts, is a closely integrated homeostatic system. When thinking about the molecular cell biology of bone modeling and maintenance, it is important to think in terms of the smallest quantum unit of bone that has all the ingredients of bone (extracellular matrix, bone matrix proteins, and mineralized matrix; see Table 1). I propose the term ''osteosome'' to signify this smallest quantum unit. The steady state mineral density of the osteosome results from the the balance of anabolic osteoblasts and catabolic osteoclasts. Osteoclasts are multinucleated and are present in both cortical and cancellous bone. The osteoclasts create a microenvironment by sealing off the cell membrane and the area of resorption. The adhesion and sealing are mediated by integrins (Hynes, 1992). The osteoclast is a polarized cell with vectorial discharge of H^+ ions to disolve minerals and enzymes such as proteases, collagenases, and cathespins to degrade the extracellular matrix osteosome (Baron, 1993). Local cytokines and systemic hormones regulate osteoclast function. The hormones are parathyroid hormone (PTH), calcitonin (CT), and vitamin D_3. The cytokines include interleukins (IL-1, IL-4, IL-6, and IL-11) and tumor necrosis factors (TNFα and TNFβ). TNF is a term coined by Lloyd Old and colleagues. It was recognized as the cachectin involved in cachexia (or wasting syndrome) by Cerami and Beutler. TNFα increases bone resorption (Bertolini et al., 1986). The molecular cloning of TNFs spawned an unprecedented period of research activity including identification of receptors. Two distinct but structurally homologous receptors for TNF, p75 and p55, were identified. As many as a dozen receptors are now known including nerve growth factor receptor, CD27, CD30, CD40, and Fas antigen (Smith et al., 1994; Aderka,

Table 1. Macromolecules in the Osteosome, the Quantum Unit of the Extracellular Matrix (ECM) of Bone

Constituent	Function
Collagens	
Type I collagen	Principal ECM
(2 α1 chains)	Component
(1 α2 chain)	Supramolecular assembly
Type III, V collagens	Minor collagens
Proteoglycans	
Versican	Occupies space due to ''extended'' conformation
Biglycan	Binds collagen
Decorin	Binds to TGFβ
Hyaluronan	Interactive with proteoglycan
Leucine-rich proteins	
Osteoadherin	Cell attachment
Osteoglycin	TGFβ/BMP binding
Noncollagenous proteins	
Osteocalcin	Remodeling
Osteonectin/SPARC	Links mineral to matrix
Osteopontin	Cell adhesion
Fibronectin	Cell adhesion
Vitronectin	Cell attachment
Thrombospondin	Multifunctional cell adhesion modulator
Bone sialoprotein	Modulator of mineralization
Bone-associated glycoprotein-75	Binds calcium
Fibrillin	Assembly of elastic fiber
Mineral	
Hydroxyapatite	Located in and on ''hole'' zones
3 $Ca_3(PO4)_2{\cdot}Ca(OH)_2$	in collagen microfibrils as plates/needles

1996). Soluble versions of these receptors bind cognate ligands and influence bioavailability. Certain soluble TNFRs are elevated in disease states such as lupus and rheumatoid arthritis.

The identification of novel regulators and signaling molecules has required approaches such as expression cloning, subtractive hybridization, candidate molecules, RT–PCR of Drosophila morphogens, and the use of expressed sequence tags (ESTs). The present identification of a novel soluble TNF receptor increasing bone density, by decreasing bone resorption, has been achieved by researchers at Amgen based on ESTs (Simonet et al., 1997). The authors of this report initially identified a novel member of the TNF receptor (TNFR) family from the fetal rat intestine. The novel cDNA encodes a 401–amino acid protein and appears to be a secreted glycoprotein with a leader sequence. The amino half of the sequence is related closely to TNFR-2 and CD40. The protein, however, lacks sequence homologies to known TNFRs in the carboxy-terminal half. Pulse-chase experiments demonstrated the dimeric nature of the secreted protein of a molecular mass of 110 kDa and is a glycoprotein. It is noteworthy that it is expressed in intestine, liver, kidney, lung, and calvaria. Calcium and phosphate transport and homeostasis is a collaborative venture of intestine, kidney, and bone. It is interesting to note that this novel gene product is highly expressed by the same triad of tissues. It is likely that there are both local and systemic functions for this protein. In humans and mouse, it is also detected in the placenta. In situ hybridization revealed localization in developing chondrogenic foci and in developing craniofacial bones.

Next, in order to assess its in vivo function, the novel rat TNF receptor–related protein was expressed under the control of human apolipoprotein E gene promoter and associated liver-specific enhancer in transgenic mice (Simonet et al., 1997). The radiographs revealed higher bone density. However, there was also splenomegaly due to occulusion of the bone marrow cavities, which resulted in compensatory extramedulary hematopoiesis. This increase in bone density was shown to be due to a decrease in the tartarate-resistant acid phosphatase (TRAP)–positive osteoclasts. Targeted disruption of the gene for TRAP also disrupts endochondral ossification and results in mild osteopetrosis (Hyman et al., 1996). Simonet et al. (1997) call this protein osteoprotegerin (OPG) because it protects bone from resorption. Direct experiments demonstrated that recombinant osteoprotegerin inhibited in vitro osteoclast differentiation. The amino-terminal domain of OPG is the business-end of the molecule with respect to restriction of the osteoclast lineage. Recombinant OPG increases bone density and protects rats against ovariectomy-induced bone loss and deficit. The human gene is localized to chromosome 8q23–24.

Many questions still need to be addressed. What is the interacting partner ligand to OPG? How and where does OPG act to restrict and to inhibit the osteoclast lineage? What are the relative roles of OPG in protecting cortical and cancellous bone? How does osteoprotegerin protect the osteosome, the smallest supramolecular unit of bone (Table 1), the quantum unit of bone, from resorption? I predict the answers should arrive in time for Christmas/Hanukkah as a gift from Amgen. Osteoprotegerin can be added to c-Src, c-Fos, and m-Csf, as another regulator of osteoclast differentiation and function.

Bisphosphonates are structural analogs of pyrophosphate that contain phosphorus-carbon-phosphorus (P-C-P) bonds instead of phosphorus-oxygen-phosphorus bonds (Fleisch, 1997). Bisphosphonates bind with high affinity to bone mineral crystals and inhibit the dissolution of minerals. Furthermore the P-C-P bonds in bisphosphonates are resistant to enzymatic hydrolysis by acid and alkaline phosphatases or pyrophosphatase. These properties explain the fact that bisphosphonates act as potent inhibitors of bone resorption in vivo. As such, they have been extensively studied for possible therapeutic application in Paget's disease of bone, hypercalcemia of malignancy, and osteolytic metastases (Fleisch, 1997). Also, ovariectomy-induced bone loss can be prevented by bisphosphonates, suggesting therapeutic use in osteoporosis. Because osteoprotegerin also blocks bone resorption in vivo, it is conceivable that osteoprotegerin may also have a clinical utility in osteoporosis. Only time and more clinical studies will tell if osteoprotegerin will be another winner, such as erythropoietin, for Amgen.

Selected Reading

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